# Nuclease

### Week 3

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

## Summary

# 1 Digestion of T7 & Nuc

## Responsible

Aman Mebrau, Oscar Frisell, Sigrun Stulz, Bethel Tesfai Embaie

## Protocols used

- 3A Assembly protocol
- Gel purification

## Modifications and comments to protocols

Not enough Nuc samples were available and the PstI single digestion was not performed.

Table 1: Modifications to digestion reactions

| **N4** | **N - (neg)** |
|---|---|
| 2 $\mu$l DNA | 2 $\mu$l DNA |
| 0.6 $\mu$l 10x cutsmart | 0.6 $\mu$l 10x cutsmart |
| 3.4 $\mu$l dd$H_2O$ | 3.4 $\mu$l dd$H_2O$ |
| 2 $\mu$l enzyme MM | |

# Experimental setup

The digestion followed the protocol. See table below for Sample structure.

Table 2: Sample structure for T7 and Nuc 2

| **T7** | **T1** | **T2** | **T3** | **T4** | **T5** | **T -** |
|---|---|---|---|---|---|---|
| Conc 35.8 ng/$\mu$l | sd EcoRI | sd SpeI | dd | dd | dd | negative control |
| **Nuc 2** | **N1** | **N2** | **N3** | **N4** | **N -** | |
| Conc 30.05 ng/$\mu$l | dd (XbaI & PstI) | dd | dd | sd XbaI | negative control | |

# Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (1)$$

# Results and Conclusions

Gel 1: ran for too long; samples were lost
Gel 2: gel was repeated with remaining T7 digestion products

Table 3: Gel labelling

| Well | Label | Comment |
|---|---|---|
| 1 | DNA ladder | |
| 2 | T7 sd EcoRI | successful |
| 3 | T7 sd SpeI | successful |
| 4 | T7 dd | similar length; could be used for ligation |
| 5 | T7 dd | similar length; could be used for ligation |
| 6 | T7 dd | similar length; could be used for ligation |
| 7 | T7 negative control | looks right |
| 8 | ladder | |

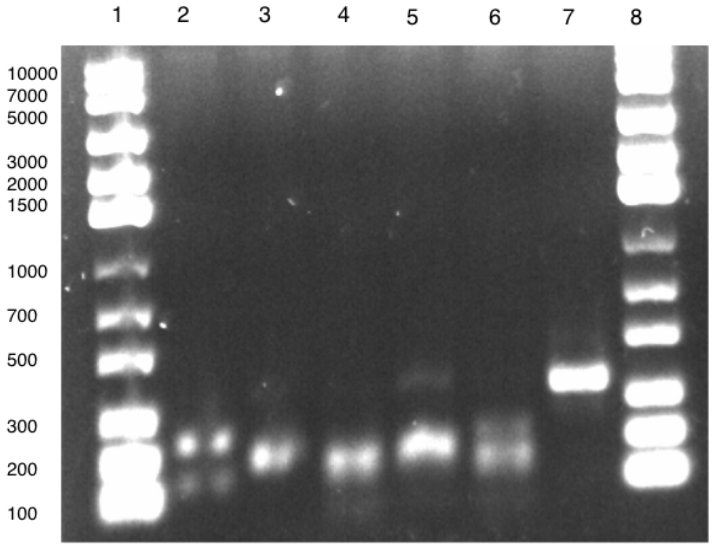


Figure 1: Successful gel of T7 digestion products.

## Discussion and Troubleshooting

The Nuc gel ran for too long so we lost it. Hence we need to amplify more Nuc and run a new gel.

# 2 PCR of Nuc from Glycerol Stocks

## Responsible

Bethel Tesfai Embaie, Oscar Frisell

## Protocols used

- PCR protocol

# Experimental Data

The aim was to amplify Nuc DNA for 3A assembly.
The total reaction volume was 50 $\mu$l for each of the 4 pcr tubes.
The table below shows how much DNA template that was used:

Table 4: Amount of DNA template per reaction

| **Label** | **DNA template [$\mu$l]** |
|---|---|
| 1 | 2 |
| 2 | 1 |
| 3 | 1 |
| 4 | 1 |

# Results and Conclusions

Table 5: Gel description

| **Well** | **Component** | **Expected bands** |
|---|---|---|
| 1 | DNA ladder | - |
| 2 | Nuc PCR product 2ul | Between 800-900 bp |
| 3 | Nuc PCR product 1ul | Between 800-900 bp |
| 4 | Nuc PCR product 1ul | Between 800-900 bp |
| 5 | Nuc PCR product 1ul | Between 800-900 bp |

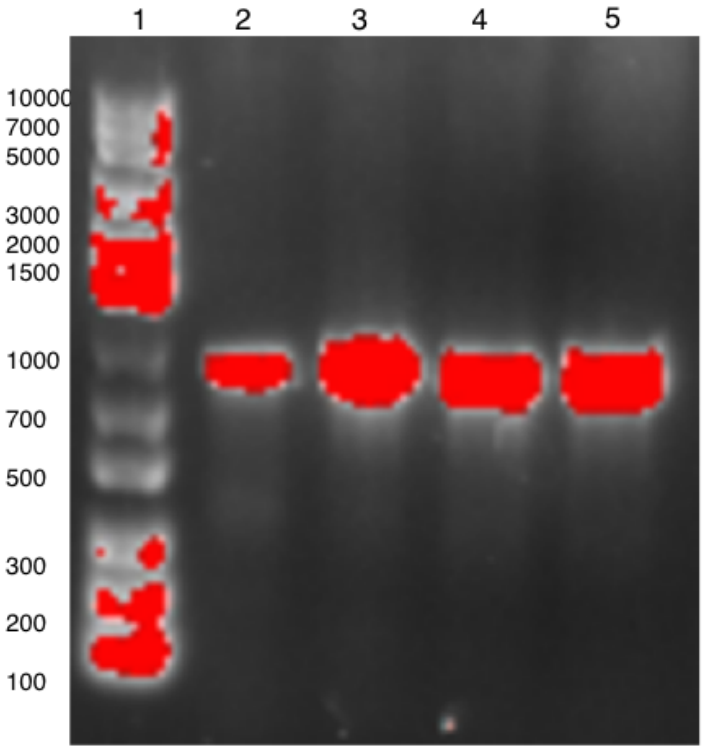


Figure 2: Gel of Nuc PCR products.

The bands in Figure 2 seem to have a size just below 1000 bp, however it is hard to tell as the bands are large and a bit smeared. Considering the expected size of between 800-900 bp, the bands might correspond to the PCR product of Nuc and thus will be PCR purified for further analysis.

## Discussion and Troubleshooting

PCR purification will be followed by NanoDrop and digestion for 3A assembly.